

CLAIMS:

Sub B1

1. An isolated nucleic acid molecule, comprising:
 - a) a sequence of nucleotides that encodes the sequence of amino acids encoded by one or more of the exons that is the complement of the sequence of nucleotides set forth in SEQ ID No. 3; or
 - b) the sequence of nucleotides set forth as one or more of the exons that are the complement of the sequence of nucleotides set forth in SEQ ID No. 3;
 - c) a sequence of nucleotides that hybridizes along its full length to the full length of at least one of the exons set forth in SEQ ID No. 3 under conditions of at least moderate stringency, and that is present in the genome of a nematode; or
 - d) a sequence of nucleotides degenerate with the sequence of nucleotides of c).
2. An isolated nucleic acid molecule, comprising:
 - a) a sequence of nucleotides that encodes the sequence of amino acids encoded by one or more of the exons that is the complement of the sequence of nucleotides set forth in SEQ ID No. 5; or
 - b) the sequence of nucleotides set forth as one or more of the exons that is the complement of the sequence of nucleotides set forth in SEQ ID No. 5;
 - c) a sequence of nucleotides that hybridizes along its full length to the full length of at least one of the exons of SEQ ID No. 5 under conditions of at least moderate stringency, and that is present in the genome of a nematode; or
 - d) a sequence of nucleotides degenerate with the sequence of nucleotides of c).
3. An isolated nucleic acid molecule of claim 1, that encodes LOV-1 protein from a nematode.
4. An isolated nucleic acid molecule of claim 2, that encodes a PKD-2 protein from a nematode.

sub B² 5. ~~The isolated molecule of claim 1 that comprises a sequence of nucleotides that encodes the amino acids set forth in SEQ ID No. 4.~~

6. The isolated molecule of claim 2 that comprises a sequence of nucleotides that encodes the amino acids set forth in SEQ ID No. 6.

5 7. The isolated nucleic acid molecule of claim 1, wherein the nematode is *Caenorhabditis elegans*.

8. The isolated nucleic acid molecule of claim 2, wherein the nematode is *Caenorhabditis elegans*.

sub B³ 9. ~~An isolated gene, comprising the nucleic acid molecule of~~
10 ~~claim 1.~~

10. The gene of claim 9, wherein the gene comprises transcriptional control sequences that are homologous to the encoded gene.

11. The gene of claim 9, wherein the gene comprises
15 transcriptional control sequences that are heterologous to the encoded gene.

12. An isolated gene, comprising the nucleic acid molecule of claim 2.

13. The gene of claim 12, wherein the gene comprises
20 transcriptional control sequences that are homologous to the encoded gene.

14. The gene of claim 12, wherein the gene comprises transcriptional control sequences that are heterologous to the encoded gene.

sub B⁴ 25 15. ~~An isolated nucleic acid molecule that encodes a mutant of the protein encoded by the nucleic acid molecule of claim 3.~~

16. The nucleic acid molecule of claim 15, wherein the mutant is a deletion mutant, insertional mutant or comprises a point mutation.

17. The nucleic acid molecule of claim 15, wherein the encoded
30 protein is inactive.

18. An isolated nucleic acid molecule that encodes a mutant of the protein encoded by the nucleic acid molecule of claim 4.

19. The nucleic acid molecule of claim 18, wherein the mutant is a deletion mutant, insertional mutant or comprises a point mutation.

5 20 17. The nucleic acid molecule of claim 18, wherein the encoded protein is inactive.

21 18. A construct comprising a nucleic acid molecule of claim 1 operatively linked to a reporter gene.

Sub 1 22 19. The construct of claim 18, wherein the reporter gene encodes a fluorescent protein.

23 20. A construct comprising a nucleic acid molecule of claim 2 operatively linked to a reporter gene.

24 21. The construct of claim 20, wherein the reporter gene encodes a fluorescent protein.

15 25 22. A plasmid, comprising a nucleic acid molecule of claim 1.

80A22 26 23. The plasmid of claim 22 that is an expression vector.

Sub B5 27 24. A transgenic nematode, comprising the vector of claim 23.

28 25. The transgenic nematode of claim 24, wherein in the vector is maintained extrachromosomally.

Sub B6 29 26. The transgenic nematode of claim 24, wherein in the vector or a gene-encoding portion is integrated into the *C. elegans* genome.

30 27. The transgenic nematode of claim 24, wherein the vector further comprises nucleic acid encoding a reporter gene operatively linked to the nucleic acid molecule.

Sub B7 25 31 28. The transgenic nematode of claim 24, wherein the nucleic acid molecule encodes a mutant protein.

32 29. The transgenic nematode of claim 27, wherein the nucleic acid molecule encodes a mutant protein.

33 30. A plasmid, comprising a nucleic acid molecule of claim 2.

30 34 31. The plasmid of claim 30 that is an expression vector.

35 32. A transgenic nematode, comprising the vector of claim 31.

36 ~~33~~. The transgenic nematode of claim ~~32~~³⁵, wherein in the vector is maintained extrachromosomally.

37 ~~34~~. The transgenic nematode of claim ~~32~~³⁵, wherein in the vector or the gene-encoding portion is integrated into the *C. elegans* genome.

5 38 ~~35~~. The transgenic nematode of claim ~~32~~³⁵, wherein the vector further comprises nucleic acid encoding a reporter gene operatively linked to the nucleic acid molecule.

39 ~~36~~. The transgenic nematode of claim ~~32~~³⁵, wherein the nucleic acid molecule encodes a mutant protein.

10 40 ~~37~~. The transgenic nematode of claim ~~35~~³⁷, wherein the nucleic acid molecule encodes a mutant protein.

41 ~~38~~. An isolated nucleic acid molecule, comprising a sequence of nucleotides encoding a mutant LOV-1 protein, wherein a nematode that expresses such defect exhibits one or both of an altered location of vulva (Lov) and response phenotype, and the LOV-1 protein is encoded by the nucleic acid molecule of claim 1.

5168 42 ~~39~~. A transgenic nematode, comprising the nucleic acid molecule of claim ~~38~~⁴¹.

43 ~~40~~. An isolated nucleic acid molecule, comprising a sequence of nucleotides encoding a mutant PKD-2 protein, wherein a nematode that expresses such defect exhibits one or both of an altered Lov and response phenotype, and the PKD-2 protein is encoded by the nucleic acid molecule of claim 2.

44 ~~41~~. A transgenic nematode, comprising the nucleic acid molecule of claim ~~40~~⁴³.

45 ~~42~~. An isolated polypeptide encoded by the nucleic acid molecule of claim 1.

46 ~~43~~. The polypeptide of claim ~~42~~⁴⁵ that comprises the sequence of amino acids set forth in SEQ ID No. 4.

30 47 ~~44~~. An isolated polypeptide encoded by the nucleic acid molecule of claim 2.

⁴⁸ 45. The polypeptide of claim ⁴⁷ 44 that comprises the sequence of amino acids set forth in SEQ ID No. 6.

⁴⁹ 46. An isolated nucleic acid molecule of claim ²² 19, comprising a sequence of nucleotides that encodes the sequence of amino acids set forth in SEQ ID No. 15.

⁵⁰ 47. An isolated complex comprising a nematode PKD-2 protein and a nematode LOV-1 protein in operative linkage.

⁵¹ 48. A method, comprising:
introducing a mutation into the *lov-1* and/or *pkd-2* gene of a
10 nematode, and
selecting nematodes that exhibit altered mating behavior, wherein the altered behavior includes a change in the ability to locate the vulva (Lov) of a hermaphrodite or a change in the response of the male to contact with the hermaphrodite (Response).

15 ⁵² 49. The method of claim ⁵¹ 48, wherein the altered behavior is a change in the response of the male to contact with the hermaphrodite.

⁵³ 50. The method of claim ⁵¹ 48, wherein the mutation is in the *lov-1* gene.

⁵⁴ 51. The method of claim ⁵¹ 48, wherein the mutation is in the
20 *pkd-2* gene.

⁵⁵ 52. The method of claim ⁵¹ 48, wherein the nematode is a species of *Caenorhabditis*.

⁵⁶ 53. A method, comprising:
treating nematodes with a test compound or with a
25 mutagenizing agent or treatment; and
selecting from among the nematodes or offspring thereof, nematodes that exhibit altered mating behavior compared to prior to the treatment; where the altered behavior includes one or both of location of vulva (Lov) or response of the male to contact with the hermaphrodite
30 (Response).

57 ~~54~~. The method of claim ⁵⁶~~53~~, wherein prior to treatment the nematodes had exhibited normal mating behavior.

58 ~~55~~. The method of claim ⁵⁶~~53~~, wherein prior to treatment the nematodes had exhibited defects in mating behavior, wherein the defects were manifested as a defect in one or both of Lov and Response, and the alteration comprises a partial restoration or complete restoration of one or both of Lov and Response behaviors.

59 ~~56~~. A method for identifying compounds, comprising:
contacting nematodes with a test compound,
selecting test compounds that result in altered mating behavior,
wherein:

the altered mating behavior comprises alteration in the behavior involving location of vulva and/or response to contact with the hermaphrodite; and

the selected test compounds are candidates for treatment of polycystic kidney diseases of mammals.

60 ~~57~~. The method of claim ⁵⁹~~56~~, wherein prior to treatment the nematodes had exhibited normal mating behavior.

61 ~~58~~. The method of claim ⁵⁹~~56~~, wherein prior to treatment the nematodes had exhibited defects in mating behavior, wherein the defects were manifested as a defect in one or both of Lov and Response, and the alteration comprises a partial restoration or complete restoration of one or both of Lov and Response behaviors.

62 ~~59~~. The method of claim ⁵⁹~~56~~, wherein the selected compounds are candidate therapeutic agents for treatment of autosomal dominant polycystic kidney disease (ADPKD) or other diseases involving PKD1 or PKD2.

63 ~~60~~. The method of claim ⁶²~~59~~, wherein prior to treatment the nematodes had defects in mating behavior, and the candidate compounds restore or partially restore either or both Lov and Response.

64 61. A method for identifying genes that are part of the disease pathway of autosomal dominant polycystic kidney disease (ADPKD), comprising:

- 5 mutagenizing nematodes that exhibit normal mating behavior; and
 identifying and selecting nematodes or the male offspring thereof that exhibit altered mating behavior, wherein the altered mating behavior comprises alteration in the behavior involving location of vulva (LOV) and/or response to contact with the hermaphrodite (Response), thereby identifying nematodes that contain defects in genes in the pathway that
 10 comprises the *lov-1* and/or *pkd-2* gene(s).

65 62. The method of claim 61, further comprising, mapping the mutation(s) in selected nematodes that results in the altered behavior.

- 66 63. The method of claim 62, further comprising, identifying
 15 mammalian homologs or orthologs of the nematode genes to which the mutation is mapped.

67 64. A method for identifying compounds that are candidate therapeutic agents for treatment of autosomal dominant polycystic kidney disease (ADPKD), comprising:

- treating male nematodes that can sire cross-progeny with moving
 20 partners with a test compound; and

 selecting compounds that result in males that sire fewer cross progeny or cannot sire cross-progeny with moving partners, wherein the selected compounds are candidate therapeutic agents for treatment of ADPKD or diseases involving PKD1 or PKD2.

- 25 68 65. A method for identifying genes that are part of the disease pathway of autosomal dominant polycystic kidney disease (ADPKD), comprising:

 mutagenizing males nematodes that can sire cross-progeny with moving partners with a test compound;

selecting males or the offspring thereof that sire fewer cross-progeny with moving partners; and

identifying the mutant nematode genes.

69 66. A method for identifying genes or regulatory factors involved
5 in polycystic kidney diseases, comprising:

mutagenizing nematodes that exhibit altered mating behaviors because of a mutation in the *lov-1* or *pkd-2* gene;

selecting nematodes or the offspring thereof that exhibit a restoration of the behavior associated with the wild-type gene; and

10 identifying a second gene other than *lov-1* or *pkd-2* or a factor that results in restoration of the behavior, wherein restoration of the behavior is a partial or complete restoration compared to prior to mutagenesis.

70 67. The method of 66, further comprising:

15 identifying a mammalian gene that is orthologous to the second gene.

71 68. A method for screening compounds to identify candidates for treatment of polycystic kidney diseases, comprising:

contacting nematodes that exhibit altered mating behaviors because of a mutation in the *lov-1* or *pkd-2* gene with a test compound;

20 and

selecting compounds that result in restoration of the behavior, wherein restoration of the behavior is a partial or complete restoration compared to prior to contacting.

72 69. A method for identifying genes or regulatory factors involved
25 in polycystic kidney diseases, comprising:

mutagenizing nematodes that exhibit altered mating behaviors because of a mutation in the *lov-1* or *pkd-2* gene;

30 selecting nematodes or offspring thereof that cannot sire cross progeny or sire fewer cross progeny with paralyzed hermaphrodite mating partners; and

identifying a gene responsible for the inability to sire cross progeny with paralyzed hermaphrodite mating partners.

73 70. The method of claim ⁷²69, further comprising identifying mammalian homologs of the gene responsible for the inability to sire cross progeny with paralyzed hermaphrodite mating partners.

5 ~~Sub B¹⁰~~ 74 71. A method for identifying genes or regulatory factors involved in polycystic kidney diseases, comprising:

mutagenizing transgenic nematodes that contain a dominant negative *lov-1* or *pkd-2* transgene;

10 selecting nematodes or offspring thereof that exhibit a further loss in function of the *lov-1* or *pkd-2* transgene by observing mating behaviors; and

identifying the mutations and genes responsible for the loss.

75 72. The method of claim ⁷⁴71, further comprising identifying homologous mammalian genes.

15 ~~Sub B¹¹~~ 76 73. A method for identifying regulators and factors necessary for synthesis and transport of *LOV-1* or *PKD-2* protein;

preparing a transgenic nematode that expresses a detectable marker linked to *LOV-1* or *PKD-2* protein;

20 mutagenizing the nematode;

selecting nematodes or offspring thereof that have altered patterns of expression of *LOV-1* or *PKD-2*; and

identifying the gene responsible for the alteration.

77 74. A method for identifying transcriptional regulators of *lov-1* or *pkd-2*; comprising:

25 preparing a transgenic nematode that expresses a detectable marker linked to *LOV-1* or *PKD-2* protein;

mutagenizing the nematode;

30 selecting nematodes or offspring thereof that altered levels of expression of the protein.

78 75. A method, comprising:

treating nematodes with a test compound or mutagenizing them;

selecting nematodes or the offspring thereof that exhibit altered clumping behavior when seeded on a lawn of bacteria, wherein:

- 5 an alteration in the behavior is indicative of change in the genotype of the *lov-1* or *pkd-2* locus;

the wild-type males exhibit clumping behavior, and a males with a mutation in either locus that alters activity of either the LOV-1 or PKD-2 protein results in males that are randomly dispersed in the bacterial lawn.

- 10 ⁷⁹ 76. The method of claim ⁷⁸ 75, wherein:

the nematodes are mutant nematodes that are randomly dispersed in the bacterial lawn and are treated with a test compound; and the method further comprises:

- 15 identifying compounds that restore or partially restore clumping behavior.

⁸⁰ 77. The method of claim ⁷⁹ 76, wherein the mutant nematodes comprise males that are *lov-1* mutants.

⁸¹ 78. The method of claim ⁷⁹ 76, wherein the mutant nematodes comprise males that are *pkd-2* mutants.

⁸² 79. The method of claim ⁷⁸ 75, wherein:

the nematodes are mutant nematodes that are randomly dispersed in the bacterial lawn and then mutagenized; and the method further comprises:

- 25 selecting males or the offspring thereof that exhibit a partial or complete restoration of the behavior;
analyzing the mutations; and
identifying the genes or mutations responsible for the restoration.

⁸³ 80. The method of claim 76, wherein the genes or mutations are genetic suppressors of *lov-1* or *pkd-2* mutants.

⁸⁴ 81. The method of claim 76, wherein the mutant nematodes comprise males that are *lov-1* mutants.

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85 ~~82.~~ The method of claim 76, wherein the mutant nematodes comprise males that are *pkd-2* mutants.

86 ~~83.~~ The method of claim ~~75~~⁷⁸, wherein:

the nematodes are wild-type nematodes that are clumped in the
5 bacterial lawn and are treated with a test compound; and the method further comprises:

identifying compounds that destroy the clumping behavior.

87 ~~84.~~ The method of claim ~~75~~⁷⁸, wherein:

the nematodes are wild-type nematodes that are clumped in the
10 bacterial lawn and then mutagenized; and the method further comprises:
selecting males or the offspring there of that are randomly
dispersed on the bacterial lawn;

analyzing mutations responsible for the altered behavior; and
identifying the mutant genes.

15 ~~88~~ ~~85.~~ A mutant strain of nematode that comprises a mutation in the *lov-1* or *pkd-2* gene, whereby the resulting nematode exhibits altered mating behavior compared to the wild-type, wherein the alteration is manifested as either or both a defect in behavior involving location of vulva (LOV) and response to contact with the hermaphrodite (Response).

20 ~~89~~ ~~86.~~ The mutant strain of claim ~~85~~⁸⁸, wherein the mutation is in the *lov-1* gene, wherein the wild-type *lov-1* gene comprises:

a) a sequence of nucleotides that encodes the sequence of amino acids encoded by one or more of the exons that is the complement of the sequence of nucleotides set forth in SEQ ID No. 3; or

25 b) the sequence of nucleotides set forth as one or more of the exons that are the complement of the sequence of nucleotides set forth in SEQ ID No. 3;

c) a sequence of nucleotides that hybridizes along its full length to the full length of at least one of the exons set forth in SEQ ID
30 No. 3 under conditions of at least moderate stringency, and that is present in the genome of a nematode; or

~~d) a sequence of nucleotides degenerate with the sequence of nucleotides of c).~~

90 87. The mutant strain of claim ~~85~~⁸⁸, wherein the mutation is in the *pkd-2* gene, wherein the wild-type *pkd-2* gene comprises:

5 a) a sequence of nucleotides that encodes the sequence of amino acids encoded by one or more of the exons that is the complement of the sequence of nucleotides set forth in SEQ ID No.5; or

b) the sequence of nucleotides set forth as one or more of the exons that is the complement of the sequence of nucleotides set forth
10 in SEQ ID No. in SEQ ID No. 5;

c) a sequence of nucleotides that hybridizes along its full length to the full length of at least one of the exons of SEQ ID No. 5 under conditions of at least moderate stringency, and that is present in the genome of a nematode; or

15 d) a sequence of nucleotides degenerate with the sequence of nucleotides of c).

91 88. The method of claim ~~85~~⁸⁸, further comprising identifying mammalian homologs of the genes that comprise the mutant nematode genes.

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